Journal of Hazardous Materials 265 (2014) 47–60

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Contribution of hot spring bacterial consortium in cadmium and lead bioremediation through quadratic programming model

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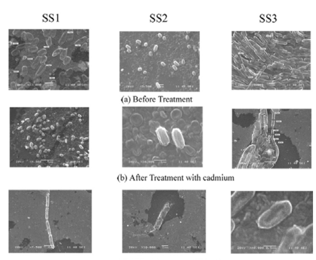
HIGHLIGHTS

- Adsorption of cadmium and lead using hot spring microbial consortium.
- Development of empirical models for % adsorption using ANOVA and response surface methodology.
- Fitting of the kinetics of adsorption to Freundlich and Langmuir model.
- Optimization of the operating parameters to maximize the % of adsorption.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 July 2013
Received in revised form
11 November 2013
Accepted 14 November 2013
Available online 22 November 2013

Keywords:
Bioremediation
Cadmium
Lead
Hot spring
Isotherm
Optimization

ABSTRACT

In the present investigation, a number of experiments have been conducted to isolate microbial strains from Taptapani Hot Spring Odisha, India for bioremediation of cadmium and lead. The strains *Stenotrophomonas maltophilia* (SS1), *Aeromonas veronii* (SS2) and *Bacillus barbaricus* (SS3) have shown better adaptation to metal tolerance test, with different concentrations of cadmium and lead and hence have been selected for further studies of metal microbial interaction and optimization. The results of bioremediation process indicate that consortium of thermophilic isolates adsorbed heavy metals more effectively than the individually treated isolates. Therefore, A 24 full factorial central composite design has been employed to analyze the effect of metal ion concentration, microbial concentration and time on removal of heavy metals with consortium. Analysis of variance (ANOVA) shows a high coefficient of determination value. The kinetic data have been fitted to pseudo-first order and second-order models. The isotherm equilibrium data have been well fitted by the Langmuir and Freundlich models. The optimum removal conditions determined for initial ion concentration was 0.3 g/l; contact time 72 h; microbial concentration, 3 ml/l; and pH 7. At optimum adsorption conditions, the adsorption of cadmium and lead are found to be 92% and 93%, respectively, and presence of metals was confirmed through EDS analysis.

1. Introduction

The production of heavy metals has been increasing with the advancement of industrialization and its toxic effect contaminating which is now a days a problem of global concern [1]. In addition to their toxic effects even at low concentrations, heavy metals can accumulate along the food chain which leads to serious ecological problem and health hazards as a result of their solubility and mobility. Lead is one such metal which has both acute and chronic effects in humans. It may cause anaemia, headache, chills, diarrhoea and reduction in haemoglobin formation. Lead poisoning causes severe damage to kidneys, nervous system, reproductive system, liver and brain [2]. Public concern about the potential of cadmium (Cd) toxicity has been increasing over the past decade, and the Codex Alimentarius Commission adopted a new international standard for Cd concentration of less than 0.4 mg kg^{-1} wet

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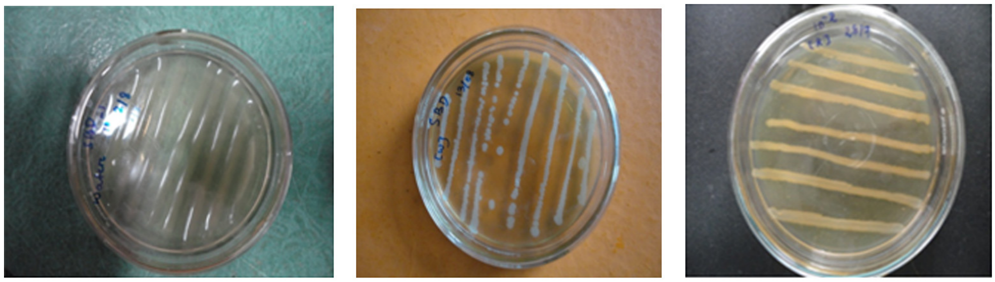
http://dx.doi.org/10.1016/j.jhazmat.2013.11.036



SS1 SS2 SS3

Fig. 1. Three different isolates from hot spring.

weight (ww) in brown rice, 0.2 mg kg^{-1} (ww) in wheat and leafy vegetables, 0.1 mg kg^{-1} (ww) in stem and tuber vegetables and 0.05 mg kg^{-1} (ww) in other vegetables [3].

The conventional methods of removing metals from wastewaters are generally expensive and have many limitations. Biosorption is the most widely used and low-cost alternative technology for heavy metal removal. In recent years there has been considerable interest in the use of sorbent materials, particularly biosorbents [4] such as wheat straw [5], bael leaves [6], pine bark [7], hyacinth [8], Tea waste [9], starch [10], agricultural by-products [11] and microbes [12]. Biomasses of bacteria such as *Pseudomonas aeruginosa* PU21 [13], *Bacillus cereus* [14], *Bacillus* sp. [15], *Enterobacter* sp. [16], *Pseudomonas putida* [17], and *Geobacillus thermodenitrificans* [18] have been found to show high absorption capacities of lead. The cell walls of the gram-negative bacterial species particularly the molecule of peptide, lipid and integral membrane protein have high content of potentially active chemisorption sites of heavy metals [19]. Biosorption of lead and the kinetics of lead removal have been studied by using nonliving biomasses [6,8,20–23]. Cadmium is one of the most dangerous heavy metals both to human health and to aquatic ecosystems having an inhibitory effect in the enzymatic defence system in hydrothermal vent mussel *Bathymodiolus azoricus* [24]. The employment of bacterial biomass for the metal removal from effluents is a perspective suggested by many researchers dealing with metal-bacteria interactions [25]. The presence on bacterial surfaces, of polarizable groups capable of interacting with cations is responsible for their reversible metal binding capacity. Such groups (sites) are mainly: phosphate, carboxyl, hydroxyl and amino-groups [26]. Although much research information is available on the single metal biosorption, relatively less attention has been paid to the biosorption of multi-metal-ion systems [27–31] and use of microbial consortium.

Literature review shows that no work has been done on bioremediation of heavy metals using hot spring microbial consortium. In the present work, it has been reported about the isolation of the microorganisms from hot spring at Taptapani, Odisha has been reported in the present work and their ability to remove cadmium and lead from wastewater. The kinetic data has been fitted to different models and the isotherm equilibrium data have been fitted to Langmuir and Freundlich isotherms.

2. Materials and methods

2.1. Sampling site and sampling procedure

Water and soil samples have been collected from Taptapani (84°40′ E and 19°50′ N) hot water spring (Odisha), India and carried in sterile plastic containers to the laboratory at ambient temperature for further analysis. The hot spring which has been studied here is located on the eastern slope of the Eastern ghat in India, Odisha, which is a hot water sulphur spring set at the greenery of a lush forest. The sulphur spring of Taptapani is situated on the top of a hill in which the hot waters erupt in bubbles in two specific places and the water temperature of this hot water varies between 40 °C and 60 °C.

One ml of water sample has been serially diluted in sterilized distilled water to get a concentration range from 10^{-1} to 10^{-6} dilution. The isolation of bacteria has been performed in enriched Luria–Bertani medium (tryptone 1%, yeast extract 0.5%, NaCl 0.5%, agar 1.5%, pH 7–7.5 at different temperatures, 37.5 °C, 50 °C and 60 °C) for 24 h. The bacterial isolates have been further sub-cultured on the respective media in order to obtain pure culture and among them three isolates (SS1, SS2 and SS3) were selected for cadmium and lead bioremediation as shown in Fig. 1. The preliminary characterization (morphological, biochemical, physiological and molecular) of the pure isolates have been carried out using existing method [32]. The details of the morphological, biochemical and physiological characterization are presented in Table 1 and the neighbour-joining phylogenetic tree from analysis of 16S rDNA gene sequence of bacterial isolated is represented in Fig. 2.

2.2. Heavy metals tolerance

Metal salts of $CdCl_2$, and ($Pb[OH]NO_3$), have been used for the study of microbial tolerance From an overnight grown culture of a single colony 1% (v/v) was transferred to 3 ml (1 ml = 7.8 × 10^2 CFU) of media supplemented with each metal separately. The initial metal concentration was taken as 1 mM. The tolerance was measured on the basis of growth observed (turbidimetry method) within 12–48 h. Based on the growth, inoculums have been added to the media with increasing concentration of the metal (0.1 mg/l, 0.2 mg/l, 0.3 mg/l, 0.4 mg/l and 0.5 mg/l) with 1 ml (50 mg on dry basis) of consortium microorganism. This step has been repeated for both the metals in each strain till the Minimum Inhibitory Concentration (MIC) was obtained as visualized by cessation of growth. Fig. 3a and b shows the percentage of cadmium and lead removal with respect to time by SS1, SS2, SS3 bacterial isolates and mixed consortium for a heavy metal ion concentration of 0.3 mg/l of solution. It is observed that the uptake capacity of mixed consortium is more than individual strains for both cadmium and lead.

2.3. Batch experiment for heavy metal adsorption

Adsorption of cadmium and lead on hot spring microorganisms have been selected containing different weighed amounts of each sample with 200 ml solution of 0.1, 0.2, 0.3, 0.4 and 0.5 mg/l of initial metal concentration. The aqueous solution of cadmium and lead have been prepared by dissolving known amount of $CdCl_2$ and $Pb[OH]NO_3$ in deionized water respectively. The capacities of hot spring isolates to adsorb cadmium and lead have been examined by measuring the initial and final concentrations of cadmium and